European Review for Medical and Pharmacological Sciences 2013; 17: 1738-1743

Effects of isoflurane in an intoxication model: experimental study

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Abstract. – BACKGROUND: Isoflurane is a volatile anaesthetic that has been commonly used since 1980. The major metabolites of isoflurane are fluoride ion and trifluoroacetate, both excreted in the urine.

AIM: This study manage to show the histopathological findings of ingested isoflurane on liver, kidney and lugs in an animal model. Twenty-one rabbits were selected and divided into three groups: Group Isoflurane-5 (I-5); Group Isoflurane-10 (I-10); and Group Control (C). Each group consisted of seven rabbits. I-5 and I-10 received 5 ml/kg and 10 ml/kg of liquid isoflurane, respectively, via nasogastric tube, while C received 5 ml/kg saline (0.9% NaCl). All animals in I-5 and I-10 were sacrificed without anesthetic drug administration. Tissue samples from livers, kidneys and lungs were collected, preserving tissue unity and avoiding infliction of any trauma. Samples were fixed in 10% formalin solution, embedded in paraffin blocks and sliced into 5 μm sections. To investigate the effects of isoflurane, sections were examined under light microscope and histopathological changes were scored.

RESULTS: Mean injury scores and the appearance of portal lymphocyte infiltration in liver samples showed significant increases in I-5 and I-10 compared to C ($p = 0.005$, $p = 0.001$ and $p = 0.001$, respectively). Mean lung injury scores revealed significant increases after isoflurane treatment in I-5 and I-10 compared to C ($p = 0.026$ and $p = 0.017$, respectively).

CONCLUSIONS: Ingested isoflurane led to mild liver and lung injuries in rabbits.

Key words:
Isoflurane, Ingestion, Drug toxicity, Histopathology, Liver, Kidney, Lung.

Introduction

Isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether; $C_3H_2ClF_5O$) is a fluorinated inhalant anaesthetic that was introduced in clinical use in 1980[1,2]. It is a clear, colourless, non-flammable, volatile liquid at room temperature and pressure. It is an isomer of enflurane and has potency between those of halothane and enflurane[2]. Isoflurane has a low blood/gas partition coefficient, which provides the advantage of requiring small volumes to enable rapid induction of anaesthesia. It produces marked respiratory depression, reduces pharyngeal reflexes and relaxes skeletal muscles on usage in anaesthetic doses[1,3]. More than 99% of inhaled isoflurane is exhaled without being metabolized. The major metabolism of isoflurane occurs in the liver, catalysed by the hepatic microsomal cytochrome P-450 enzyme system. The main identified metabolites of isoflurane are fluoride ion and trifluoroacetate, both excreted in the urine[1,4]. A systematic search of the literature revealed that several experimental studies and case reports have dealt with isoflurane toxicity by inhalation[2,5-11].

In addition, a few cases reported isoflurane being used as a suicide agent by ingestion, injection or inhalation. Only three of these cases reported isoflurane being ingested for purposes of suicide[1,12]. Nevertheless, the effects of ingested isoflurane on the liver, kidneys and lungs have not been researched. Therefore, the current study undertakes to show the histopathological findings of ingested isoflurane in an animal model.

Materials and Methods

Animals

The study was performed with the approval of the Experimental Animals Ethics Committee at the Gaziosmanpasa University Experimental and Clinical Research Center (2012 HADYEK-033; Date, 10/01/2013). The study used 21 male New Zealand white rabbits aged 17-23 weeks and weighing 3.25-4.20 kg. The rabbits received food and water *ad libitum*. They were kept in an air-conditioned animal house at a temperature of 25 ± 2 ºC and 50-60% humidity, with a 12-h light-dark cycle. The rabbits were divided into 3 groups: Group Isoflu-

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Table I. The scoring parameters of histopathological changes in liver.

	0	1	2	3
Hydrophilic degeneration	No change	In 10-20 % of the cell	In 20-50 % of the cell	In more than 50 % of the cell
Nuclear polymorphism	No change	In 10-20 % of the cell	In 20-50 % of the cell	In more than 50 % of the cell
Portal neutrophilic infiltration	No change	In 1-2 portal areas	3-5 portal areas	In more than 6 portal areas
Portal lymphocyte infiltration	No change	In 1-2 portal areas	3-5 portal areas	In more than 6 portal areas
Focal necrosis	No change	In 1-2 portal areas	3-5 portal areas	In more than 6 portal areas

rane-5 (I-5); Group Isoflurane-10 (I-10); and Group Control (C). Each group consisted of 7 rabbits. Nasogastric tubes were inserted in all rabbits before administering liquid isoflurane or saline. Groups I-5 and I-10 received 5 ml/kg and 10 ml/kg via nasogastric tube, respectively, of liquid isoflurane, while C received 5 ml/kg saline (0.9% NaCI) at the same route. The animals were observed every 5 minutes after administration. All animals in I-5 and I-10 were sacrificed without anesthetic drug administration. Tissue samples from livers, kidneys and lungs were obtained, preserving the tissue unity and avoiding infliction of any trauma. Samples were fixed in a 10% formalin solution, embedded in paraffin blocks and sliced into 5 µm sections (LeicaRM 2135; Leica Instruments; Nussloch, Germany). After the sections were dyed in haematoxylin-eosin, histopathological evaluation was performed under light microscope (LeicaDM 2500; Leica Instruments; Nussloch, Germany).

Histopathological Evaluation

Histopathological assessments of livers and kidneys were performed according to the Demirel et al[13] and Bayar et al[14] scoring systems, respectively. All sections from livers were assessed for hydropic degeneration, nuclear polymorphism, portal neutrophils infiltration, portal lymphocytes infiltration and focal necrosis. Table I presents scoring parameters of the histopathological changes in livers.

According to Bayar et al[15] all sections from kidneys were evaluated and scored as 0 = No pathological changes; 1 = Mild Injury (glomerular mesangial proliferation and interstitial venous congestion); 2 = Moderate Injury (basal membrane thickness, tubular substance accumulation and interstitial nephritis); 3 = Severe Injury (structural lipid degeneration of the cell and permanent changes in the basal membrane).

Lung injury was rated with a semi-quantitative score based on congestion, interstitial oedema, PMN (polymorphonuclear) infiltration and airspace haemorrhage, as follows: 0 = no changes; 1+ = focal, mild, subtle changes; 2+ = multifocal, mild changes; 3+ = multifocal, moderate changes; and 4+ = extensive, prominent changes.

Statistical Analysis

Normality and variance were tested using One Sample Kolmogorov-Smirnov test, skewness, kurtosis and histograms for each variable. Quantitative data were presented as means and standard deviation, and qualitative data as frequency and percentage. Depending on these results, nonparametric analysis was undertaken for each variable. Histopathological value differences among groups were analyzed by Mann-Whitney U test and Fisher's exact test. Analyses were conducted using the Statistical Package for Social Sciences program (SPSS Inc., Chicago, IL, USA), version 20.0. Statistical significance for all analyses was set at $p < 0.05$.

Results

Table II, Figure 1B and Figure 1C show the frequency of hydropic degeneration, nuclear polymorphism, portal neutrophils infiltration, portal lymphocytes infiltration, focal necrosis, hepatic injury scores and histopathological evaluation for livers in I-5 and I-10.

Figure 1B and 1C show severe portal lymphocytes infiltration in I-5 and I-10 and moderate portal neutrophils infiltration in I-10.

Based on histopathological assessment of livers, the occurrence of portal lymphocytes infiltration and mean injury scores in I-5 and I-10 were found to be significantly higher than in C (p = 0.005, p = 0.001, respectively, Table III). The frequency of histopathological changes in I-10 was higher than in C (p = 0.001). The mean lung injury scores of I-5 and I-10 were significantly higher than those in C (Table IV).

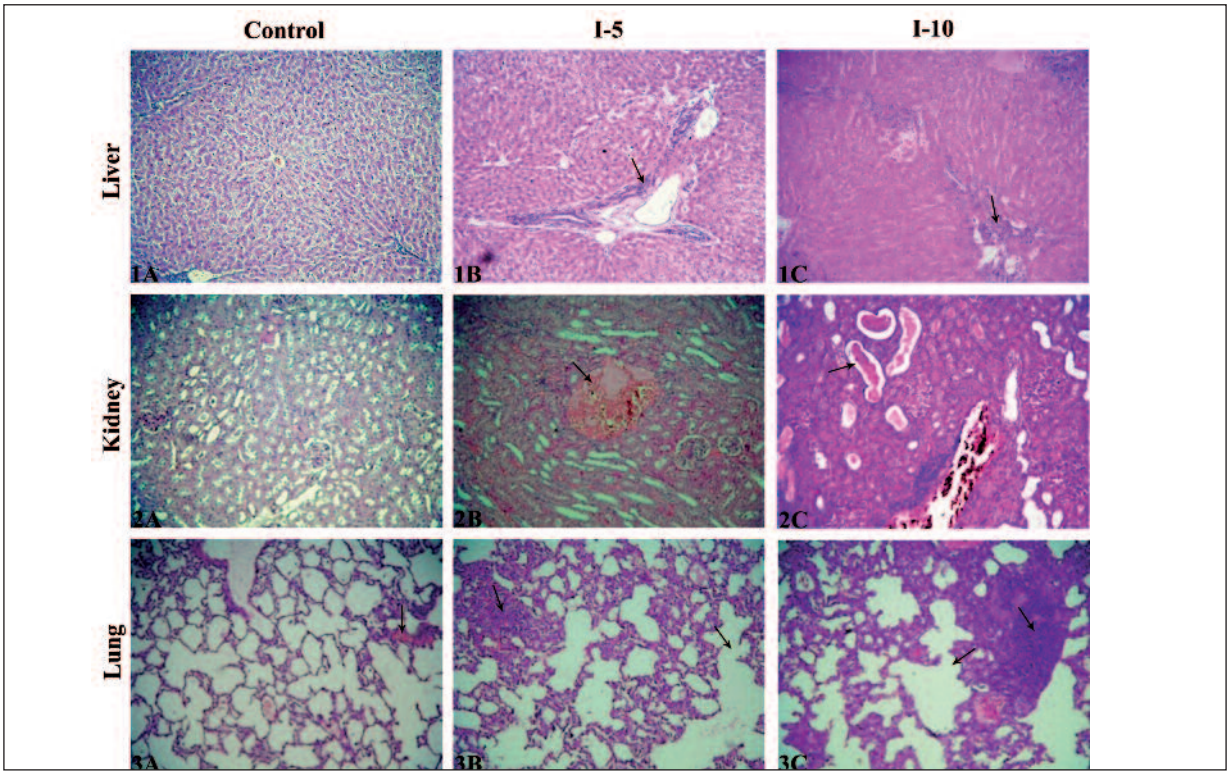

Figure 1. Representative figures of histology of rabbit liver **(1A, 1B, 1C)**, kidney **(2A, 2B, 2C)** and lung **(3A, 3B, 3C)** tissues as indicated by hematoxylin and eosin staining (x25 objective). The black arrows in **1B** indicate enlarged portal space and mild-moderate lymphocytes infiltration, in **1C** indicate moderate neutrophils, lymphocytes infiltration, and moderate nuclear polymorphism, in **2B** indicate venous congestion in interstitium, **2C** indicate substance accumulation in tubules, congestion in interstitium, and lymphocytes infiltration, in **3B** indicate congestion, oedema, neutrophils infiltration, and alveolar damage in focal areas, in 3C indicate congestion, neutophils-lymphocytes infiltration, alveolar damage in focal areas, and septal thickness in one area.

Histopathological Assessment

Portal lymphocytes infiltration in liver samples belonging to I-5 and I-10 were evident (Figure 1B, 1C). Nuclear polymorphism in hepatocytes was commonly observed in I-10 (Figure 1C). No focal necrosis was detected in any liver sample. In addition to interstitial congestion in I-5, tissue samples from kidneys in I-10 revealed substance accumulation in tubules and interstitial nephritis (Figure 2B, 2C). Oedema, congestion, focal neutrophils infiltration and areas of alveolar damage were observed in lung tissue samples in I-5 (Figure 3B). In addition to these findings, lymphocytes infiltration was detected in I-10. However, septal thickness was recognized in only one rabbit (Figure 3C).

Discussion

This study revealed that isoflurane causes substantial histopathological changes and mild or moderate tissue damage in the liver, kidneys and lungs when administered via nasogastric tube. Higher hepatic and lung injury scores were detected in rabbits that ingested isoflurane.

A systematic search of the literature showed that there has been no study, except case reports, on the toxic effects of isoflurane when ingested. Isoflurane is a volatile inhalant anesthetic agent commonly used worldwide. Various studies have been conducted to determine the effects of isoflurane; however, these studies have been focused on a single form of exposure-inhalation[16-25].

Several case reports have shown that isoflurane has toxic effects on the liver when administered by inhalation, and centrilobular necrosis in the liver generally has been reported after post-mortem pathological examinations[2,5,7,9,10,26-28]. In addition, an experimental study conducted by Fassoulaki et al[16] demonstrated that isoflurane appeared to be non-toxic or minimally toxic to the liver. Harper et al[17] and Nishiyama et al[18] reported that isoflurane did not lead to hepatic in-

Table II. The incidence of hepatic injury parameters and hepatic injury scores based on histopathological evaluation.

	C NCIHI	I-5 NCIHI	I-10 NCIHI
HD	0	2	3
NP	0	2	4
PLI	$0^{a, b}$	$6^{a, \Phi}$	$7^{b, \Phi}$
PNI	0	2	3
FN	0	0	0
TIS	0	14	23
MIS (Mean ± SD)	$0^{c, d}$	$2.00 \pm 0.57^{c, \beta}$	$3.42 \pm 1.71^{d, \beta}$

[a]: $p = 0.005$, [b]: $p = 0.001$, [c]: $p = 0.001$, [d]: $p = 0.001$; [Φ]: Fisher's Exact test, [β]: Mann-Whitney U test, NCIHI: Number of Cases Including Hepatic Injury; HD: Hydropic Degeneration, NP: Nuclear Polymorphism, PLI: Portal Lymphocytes Infiltration, PNI: Portal Neutrophils Infiltration, FN: Focal Necrosis, TIS: Total Injury Score, MIS: Mean Injury Score.

jury. In addition, the Anesthetic and Life Support Advisory Committee to the United States Food and Drug Administration (FDA) concluded that there has not been a reasonable association between isoflurane and hepatic dysfunction[30]. These results suggest that the effects of isoflurane on the liver are still in question.

Furthermore, Durak et al[19] assessed the renal effects of inhaled isoflurane on guinea pig kidneys and reported that histopathological examination revealed necrosis in proximal tubular cells, cellular residues in distal tube lumens, glomerular congestion and hypercellularity. Moreover, they concluded that isoflurane might impair enzymatic and non-enzymatic antioxidant defence systems by creating metal complexes based on elevated fluoride concentrations, resulting in oxidative stress in tubular cells and accelerated peroxidation reaction in renal tissue. However, no satisfactory explanation has been found for the molecular mechanism of fluoride nephrotoxicity[20-23].

Additionally, Molliex et al[24] and Yang et al[25] showed that isoflurane may have an effect on the metabolism of alveolar type II cells, such as increased lactate production, depressed phosphatidylcholine synthesis and induced time-dependent cellular death, which, in turn, may result in decreased lung function. Based on all these studies, it can be speculated that isoflurane has toxic effects on the liver, kidneys and lungs in inhaled form.

As mentioned above, the effects of ingested isoflurane have not been defined in detail by any controlled studies. Only three reports involve fatalities due to isoflurane abuse. In the first case report, an operating-room assistant was found dead beside an empty bottle of isoflurane. An autopsy of the deceased revealed that the highest level of isoflurane was found in the liver; gastric contents and brain tissues also had high concentrations of isoflurane. In addition, macroscopic and histological examination of the brain, heart and kidneys showed acute congestion. The report authors concluded that isoflurane restricted breathing enough to cause asphyxiation[1].

Table III. The histopathological assessment of kidney.

	C NCIRI	I-5 NCIRI	I-10 NCIRI
No Changes	$7^{a, \dagger}$	3	$1^{a, \dagger}$
Mild Injury	0	3	3
Moderate Injury	0	1	3
Severe Injury	0	0	0

[a]: $p = 0.005$; [†]: Fisher's Exact test, NCIRI: Number of Cases Including Renal Injury

The second case report consisted of two cases, the first of which was of a hospital employee found dead with a bottle of isoflurane in his hand. However, autopsy of the deceased showed presence of multiple drugs in the blood and tissues. So, investigators concluded that isoflurane was a contributing factor in the death. The second case was a research laboratory employer whose postmortem biochemical examination revealed isoflurane at a concentration of 45.9 mg/L in the blood, 97.2 mg/kg in the liver, 34.5 mg/kg in the lungs and 27.3 mg/kg in the kidneys[12]. These findings suggested that isoflurane was distributed to tissues associated with blood/tissue partition coefficients. The failing of these two case reports is that they did not perform a detailed histological examination on the samples to observe cellular changes.

Consequently, the toxic effects on the tissues of isoflurane exposure may only be speculated upon based on the findings of these case reports.

In the current study, histopathological examination of liver samples revealed increased portal lymphocytes infiltration in rabbits treated with isoflurane. This finding suggests that isoflurane entered directly into portal venous circulation after ingestion and led to acute inflammation in the portal vein area. Portal neutrohils infiltration and focal necrosis are considered histopathological signs of toxic effects on the liver[31,32]. Minimal portal neutrophils infiltration without focal necrosis would indicate that isoflurane could have a lower toxicity

Table IV. Histopathological evaluation of lung.

	C NCILI	I-5 NCILI	I-10NCILI
Edema	2	2	3
Congestion	2	4	4
Neutrophils infiltration	0	3	3
Lymphocytes infiltration	0	2	4
Alveolar damage	0	2	3
Septal thickness	0	0	1
TLIS	2	9	12
MLIS (mean±SD)	$0.28 \pm 0.48^{a,b,\gamma}$	$1.28 \pm 0.75^{a,\gamma}$	$1.71 \pm 1.11^{b,\gamma}$

[a]: $p = 0.026$, [b]: $p = 0.017$; [γ]: Mann-Whitney U test, NCILI: Number of Cases Including Lung Injury

on the liver. Lower mean injury scores of the liver in I-5 and I-10 suggest a similar conclusion.

Histopathological findings in kidneys in I-5 and I-10 revealed identical results as in Durak et al[19]. Inorganic fluoride, which is one of the metabolites of isoflurane, was blamed for creating toxicity in the kidneys. Higher fluoride levels were observed after administering increased isoflurane concentrations[33,34]. Interstitial congestion in I-5 and I-10 (mild injury), substance accumulation in tubules and interstitial nephritis in I-10 (moderate injury) suggest that direct isoflurane entry into portal venous circulation may lead to elevated metabolite levels, especially of inorganic fluoride, after elimination in the liver, which can cause tubular cell toxicity.

Isoflurane causes a decrease in alveolar type II cell Na/K-ATPase enzyme function[35]. Transepithelial Na transport helps to regulate alveolar fluid balance; therefore, corruption in the transport system may lead to decreased alveolar epithelial fluid clearance and alveolar oedema[36]. In addition, isoflurane may cause a decrease in surfactant levels because of inhibiting phosphatidylcholine synthesis and may involve damage to alveolar type II cell functions under peroxidation[24,25]. In the current study, histopathological findings suggest that alveolar type II cell damage and surfactant decrease may trigger an inflammatory response in lung tissue.

Case reports suggest that suicide and drug-related death ratios among healthcare personnel have not been underestimated. Alexander et al reported that 250 suicides per 100,000 anaesthesiologists have been estimated, a suicide rate 15 times higher than in the population at large[37-39]. In healthcare environments, it is easy to obtain isoflurane for purposes of suicide. For this reason, the exact effects of ingested isoflurane must be investigated and understood.

Conclusions

The current study suggests that isoflurane may have several mild or moderate effects on the liver, kidneys and lungs of rabbits that have ingested it. However, further studies are needed to illuminate the exact mechanisms of these effects.

Declaration of interest

All Authors declare that there is no conflict of interest.

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